Anti-LFA-1 Therapy in a Nonhuman Primate Renal Transplant Model of Costimulation Blockade Resistant Rejection

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Abstract

Costimulation blockade with the fusion protein belatacept provides a desirable side-effect profile and improvement in renal function compared to calcineurin inhibition in renal transplantation. This comes at the cost of increased rates of early acute rejection. Blockade of the integrin molecule LFA-1 has been shown to be an effective adjuvant to costimulation blockade in a rigorous non-human primate (NHP) model of islet transplantation. We therefore sought to test this combination in an NHP renal transplant model. Rhesus macaques received belatacept maintenance therapy with or without the addition of LFA-1 blockade, achieved using a murine derived LFA-1 specific antibody, TS1/22. Additional experiments were performed using chimeric, rhesus IgG1 (TS1/22R1) or IgG4 (TS1/22R4) variants, each engineered to limit antibody clearance. Despite evidence of proper binding to the target molecule and impaired cellular egress from the intravascular space indicative of a therapeutic effect similar to prior islet studies, LFA-1 blockade failed to significantly prolong graft survival. Furthermore, evidence of impaired protective immunity against CMV was observed. These data highlight the difficulties in translating treatment regimens between organ models, and suggest that the primarily vascularized renal model is more robust with regard to belatacept resistant rejection than the islet model.

Introduction

Costimulation blockade has emerged as a viable alternative to calcineurin inhibition as a base maintenance therapy for clinical renal transplantation. Specifically, use of the B7-CD28 specific fusion protein belatacept has been shown to provide similar graft survival rates, but improved graft function when compared to cyclosporine in two phase 3 registration trials, the BENEFIT (1) and BENEFIT-EXT (2) trials. However, in both trials, gains in renal function were balanced against increased rates of early acute cellular rejection. Additionally,
these rejection episodes were often more severe than early rejection seen in the cyclosporine arm of the study. A meta-analysis of 4 clinical trials revealed an overall acute rejection rate of just over 25%, and a somewhat higher rate has been anticipated outside controlled trials (3). While these data have tempered the enthusiasm for costimulation blockade-based regimens, the promise of calcineurin inhibitor-free immunosuppression has prompted a large volume of work to understand costimulation blockade-resistant rejection and find agents that may synergize with costimulation blockade to reduce early rejection without negatively impacting the long-term benefits.

Integrin molecules have long been established to play an important role in immune cell homing and trafficking to sites of inflammation or infection. Perhaps the best characterized of these molecules is the L2 integrin, Leukocyte Function-associated Antigen (LFA)-1. LFA-1 is known to mediate leukocyte adherence to the capillary endothelium though its binding to ICAM-1 (4), help stabilize the immune synapse (5), and pass bidirectional signals across the cell membrane (6). The importance of LFA-1 to the overall function of the immune system is highlighted by leukocyte adhesion deficiency (LAD), the clinical syndrome associated with its deficiency, which is characterized by recurrent bacterial infections and an inability to form abscesses. Importantly, LFA-1 expression has been shown to increase with antigen experience, and be highly prevalent on memory T cells (7), cells that have been shown to have reduced expression of CD28 (8).

The addition of LFA-1 blockade to costimulation blockade has been shown to improve the latter’s rejection profile without reverting to calcineurin inhibition. Specifically, the mouse antibody TS1/22 has been paired with belatacept in a NHP model of intraportal pancreatic islet allotransplantation with remarkable prolongation of survival (7). This observation was further supported by the report of positive outcomes in a human islet study using the humanized LFA-1-specific agent, efalizumab (9). Unfortunately, efalizumab was removed from the market following the development of progressive multifocal leukoencephalopathy (PML) in a small number of patients taking the drug for psoriasis.

Given the promising results in islet transplantation, we sought to apply the combination of LFA-1 blockade to belatacept in a NHP renal allotransplantation model and assess the relevance of this strategy to primarily-vascularized, solid organ transplants. As LFA-1 is thought to impede leukocyte diapedesis, its efficacy cannot be assumed to be similar between cellular and solid organ models of transplantation. In particular, solid organ transplants are characterized by donor endothelial activation promoting ICAM-1 upregulation, and this dynamic alteration of the LFA-1 binding ligand could reasonably be presumed to alter the response to LFA-1 blockade. Based on the previous success of TS1/22, we chose to continue with this agent. Additionally, we tested two chimeric primatized variants of the antibody in an effort to reduce any possible anti-drug responses. We show that LFA-1 blockade does not provide significant benefit to graft survival when added to a belatacept-based regimen in renal transplantation. Furthermore, our data add to the growing concerns regarding the deleterious effects of LFA-1 blockade on protective immunity. Importantly, this study highlights the differences between islet and renal transplant models and demonstrates the renal allotransplantation model provides a robust costimulation blockade-resistant response against which new therapies can be tested.
Materials and Methods

The care and treatment of all experimental animals in this study was conducted with the approval of the Emory University Institutional Animal Care and Use Committee and in adherence with the principles laid out in The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS. The veterinary staff examined all animals on a regular basis and was immediately available for consultation. Rhesus macaques (Macaca mulatta) were obtained from breeding colonies at AlphaGenesis, Inc. (Yemassee, SC, USA) or Yerkes National Primate Research Center (Lawrenceville, GA, USA). All animals underwent class I and class II MHC typing by 454 pyrosequencing (University of Wisconsin, Madison, WI, USA). Donor-recipient pairs were selected based on size matching and maximization of MHC disparity. Transplantation was performed in a domino fashion to maximize the utility of the available animals, with each animal serving as a kidney donor prior to receiving a transplant. Left donor nephrectomy was performed at least 3 weeks prior to transplantation. Renal transplantation was performed as previously described (10), and a right nephrectomy was simultaneously conducted to leave each animal entirely dependent on its allograft. Post-transplant monitoring consisted of daily clinical assessment by veterinary staff, as well as laboratory studies, including serum chemistry and complete blood count assessments performed at least weekly, or more often as dictated by the animal’s clinical course.

Generation and production of anti-LFA-1 antibodies

Mouse anti-human anti-LFA1 antibody, TS1/22, was produced from the hybridoma (HB-202, American Type Culture Collection, ATCC, Vienna, VA) in serum free medium. Mouse-rhesus chimeric forms of this antibody were generated by cloning the immunoglobulin variable region genes using 5′ rapid amplification of cDNA ends-polymerase chain reaction. The immunoglobulin heavy and light chain variable regions were subcloned into expression vectors containing rhesus IgG1 or rhesus IgG4 heavy chain and rhesus kappa light chain constant region sequences. Recombinant heavy and light chains were subcloned into expression vectors and packaged in retroviral vectors used to transduce Chinese hamster ovary cells using the GPEx™ expression technology (Catalent Pharma Solutions, Madison, WI, USA). A pool of transduced cells was grown in serum-free medium. Secreted mouse or recombinant antibody was purified by protein A affinity chromatography. The purified mouse (TS1/22), chimeric rhesus IgG1 (TS1/22R1) and IgG4 (TS1/22R4) antibodies were diafiltered into phosphate buffer. Endotoxin levels were confirmed to be less than 1 EU/mg.

Experimental Groups and Immunosuppression

This study was comprised of four experimental groups (Figure 1). The first group of 3 animals received only the base regimen, and was not exposed to anti-LFA-1 therapy. This group received a single intraoperative dose of methylprednisolone (15mg/kg) and belatacept (Nulojix; Bristol-Myers-Squibb, New York, NY, USA) maintenance therapy consisting of 20mg/kg on days 0, 3, 7, 14 and then every 14 days until day 182 when doses were spaced to every 28 days.
Anti-LFA-1 therapy was provided, in addition to the base regimen above, in the three remaining groups. The second experimental group consisted of 4 animals treated with tapering doses of TS1/22: 20mg/kg on days 0 and 3; 10mg/kg on days 7, 10 and 14; and then 5mg/kg twice weekly until day 59. This dosing is identical to that used in prior islet transplantation studies (7). TS1/22 is a mouse monoclonal antibody specific for the CD11a subunit of human LFA-1 (11). To reduce the possibility of anti-drug responses, we additionally tested mouse-rhesus chimeric versions of TS1/22 as rhesus IgG1 (TS1/22R1) or rhesus IgG4 (TS1/22R4) variants. The third and fourth experimental groups of 3 and 4 animals per group received the R1 and R4 antibodies, respectively. Dosing was identical to the group that received the fully mouse antibody.

Flow Cytometry

Analysis of circulating immune cell phenotypes was performed both prior to transplant and at regular intervals following transplantation. Data from flow cytometric analysis was combined with complete blood counts to calculate total numbers of circulating T cells and various T cell subsets. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density centrifugation (BD Biosciences, Franklin Lakes, NJ) within 6 hours of phlebotomy. These fresh PBMCs (1.5x10^6) were incubated with antibody mixtures at the appropriate titer for 15 minutes then washed twice. Following surface staining, cells were fixed and permeabilized with BD Cytofix/Cytoperm (BD Biosciences) according to the manufacturer’s direction. Flow cytometric data was acquired immediately using a BD LSR II multicolor flow cytometer (BD Biosciences). All flow data was analyzed using FlowJo (Tree Star, San Carlos, CA).

Surface markers were stained with the following monoclonal antibodies (mAbs): CD3 PacBlue, CD3 APC-Cy7, CD4 PerCP-Cy5.5, CD8 V500, CD28 PE-Cy7, CD127 PE-Cy7, CD11a APC, CD20 APC-Cy7 (all BD Biosciences), CD95 PacBlue, CD69 FITC (Invitrogen, Grand Island, NY), and CD25 PE (Miltenyi Biotech, San Diego, CA). Intracellular staining for FoxP3 was performed using FoxP3 Alexa488 (Biolegend, San Diego, CA).

Histology and Immunohistochemistry

Renal grafts procured at the time of rejection were fixed in 10% formalin, dehydrated, and embedded in paraffin. The paraffin blocks were cut into 5 μm-thick sections and underwent Hematoxylin and eosin (H&E) staining for histology assessment. Representative images were taken using Olympus BX43 microscopy.

Viral Monitoring

To mimic the clinical scenario with regard to viral prophylaxis, we gave all animals prophylaxis against rhesus Cytomegalovirus (rhCMV) with valganciclovir (60mg PO daily). To assess the effect of LFA-1 blockade on protective immunity, weekly monitoring of rhCMV viral loads was performed by quantitative real-time polymerase chain reaction using DNA isolated from whole blood as previously described (12). Titers of greater than 10,000 copies/mL were considered significant and were treated with conversion to ganciclovir (6mg/kg IM twice daily) until resolution of the viremia.
Statistics

All statistical analyses were performed in Prism 5 (GraphPad Software, La Jolla, CA, USA). Survival statistics were calculated using the Mantel-Cox method. Comparisons of cell frequency and surface expression of LFA-1 were conducted using an unpaired Student’s t test. For all analyses, a two-tailed p-value of < 0.05 was considered statistically significant.

Results

LFA-1-specific antibodies Bind to Rhesus CD11a

To confirm the ability of mouse TS1/22 to bind rhesus LFA-1, PBMCs isolated from treated animals were stained with an anti-CD11a antibody and analyzed by flow cytometry. The mean fluorescent intensity (MFI) of CD11a antibody on CD3+ lymphocytes was measured. Compared to animals receiving belatacept without LFA-1 blockade, animals receiving TS1/22 showed decreased MFI of CD11a at 7 and 14 days (p = 0.005 and 0.002, respectively, Figure 2). By 21 days, the difference between the two groups was no longer statistically significant (p = 0.77). Similar analysis was used to evaluate the binding of the chimeric forms of TS1/22. Animals treated with the R1 or R4 variants demonstrated similar decreases in CD11a MFI compared to the control group. This decrease remained statistically significant to 28 days (p = 0.02 and 0.01 for R1 and R4, respectively), suggesting a modestly improved biological half-life, consistent with the intended design of the antibodies.

LFA-1 Blockade Induces Peripheral Lymphocytosis

A prominent therapeutic effect of LFA-1 blockade is lymphocyte diapedesis inhibition, which manifests as peripheral lymphocytosis. To ensure that the LFA-1 blockade was having its intended effect, we tracked peripheral blood counts for all animals in this study. Compared with animals receiving belatacept alone, animals receiving the combination of belatacept and LFA-1 blockade developed a marked peripheral lymphocytosis by post-transplant day 3 (Figure 3A). This persisted until post-transplant day 28 before returning to baseline in animals that had not rejected by this point. Flow cytometric analysis revealed that this lymphocytosis was composed primarily of CD3+ lymphocytes (Figure 3B) and did not differ between the varying LFA-1-specific antibodies.

LFA-1 Blockade Does Not Affect the Relative Abundance of Memory or Regulatory T Cell Populations

To assess the effect of LFA-1 blockade on memory and regulatory T cells, flow cytometric analysis of T cell subpopulations was performed weekly. No difference was seen between treatment groups in the kinetics of naïve (T_N), central memory (T_CM), and effector memory (T_EM) subsets of CD8+ (Figure 4) or CD4+ (Data not shown) T cells. During treatment, a gradual decline in CD4+ regulatory T cells was seen, with similar kinetics in all treatment groups (Figure 5).
Transient LFA-1 Blockade Does Not Extend Kidney Allograft Survival When Used With Belatacept, but Promotes CMV Reactivation

Rejection free survival was measured for all animals (Table 1). Rejection was defined as time to doubling of baseline creatinine (Figure 6) and histology consistent with rejection at the time of animal sacrifice (Figure 7). One animal developed graft loss on post-operative day 1 attributable to arterial thrombosis found at necropsy, and was excluded from analysis. Concomitant treatment with TS1/22, TS1/22R1, or TS1/22R4 did not prolong rejection-free survival beyond what was seen with belatacept alone (Figure 8). While some animals achieved long-term survival, elevations in serum creatinine and rejection were noted to occur with resolution of peripheral leukocytosis and decreasing doses of TS1/22 in most LFA-1 blockade animals (Figure 9).

rhCMV viral loads were measured by PCR each week. Animals receiving the combination of belatacept and LFA-1 blockade had persistent low-level rhCMV reactivations despite anti-viral prophylaxis (Figure 10). Two animals receiving mouse TS1/22 had viral loads exceeding the pre-determined threshold of 10,000 copies/mL. These animals were switched from oral valganciclovir to IV ganciclovir until resolution of their viremia, at which time they returned to oral prophylaxis. No animal developed clinical signs or symptoms of rhCMV disease. These data, combined with the binding and lymphocytosis data, suggest that all three TS1/22 variants mediated an LFA-1 blockade biological effect, but that this effect failed to synergize with belatacept in delaying renal allograft survival.

Discussion

Costimulation blockade with belatacept provides an attractive alternative to calcineurin inhibition as a primary maintenance immunosuppressant in renal transplantation. However, the increased rates of early rejection seen in trials of belatacept have prevented its widespread acceptance. LFA-1 is an integrin molecule known to be important in lymphocyte adhesion and migration and plays a significant role in stabilization and signaling of the immune synapse. Previous work has shown the combination of belatacept and LFA-1 blockade using the monoclonal antibody TS1/22 to be effective in prolonging pancreatic islet allograft survival (7). We sought to test the generalizability of this regimen to solid organ transplantation using a renal transplant model. We found that the combination of B7-CD28 blockade and LFA-1 blockade did not prolong allograft survival beyond that seen with B7-CD28 blockade alone.

LFA-1 plays an important but non-exclusive role in the arrest and adhesion of lymphocytes rolling along the endothelial surfaces of blood vessels, ultimately allowing for migration of these cells into the tissue. This is of particular importance in the high endothelial venules (HEVs) of lymph nodes (13). The ability of circulating lymphocytes to enter the lymph node via these HEVs and survey antigen presenting cells within the lymph node is a critical step in initiating the adaptive immune response. The peripheral leukocytosis observed in this study, as well as previous islet studies, is likely multifactorial. One possibility is the lymphocytes exposed to LFA-1 blockade are impaired with regard to their ability to exit the vascular space into lymph nodes and other peripheral tissues. However, our data suggest that there is significant redundancy in this function. In particular, the 4 Integrin, very late
antigen (VLA)-4 is also capable of arresting rolling leukocytes. Previous studies have shown, like LFA-1, increased surface expression of VLA-4 on activated memory T cells (14), and these cells are thought to be involved in costimulation blockade-resistant rejection due to their decreased requirement for CD28 signaling (15). Additionally, VLA-4 may be the more important molecule for trafficking of lymphocytes into the allograft itself (16), where peripheral antigen presentation may take place. Experiments conducted in a mouse model of costimulation blockade-resistant rejection suggest, similar to LFA-1 blockade, VLA-4 blockade is capable of suppressing some aspects of the immune response (17). Studies combining the anti-VLA-4 agent, natalizumab, with belatacept in a non-human primate renal transplant model are ongoing.

Treatment with LFA-1 blockade did not appear to alter the relative abundance of memory and regulatory T cells in this study. Memory populations remained relatively stable, and treatment with TS1/22 did not deplete or augment any one population preferentially, consistent with our previous experience (7). Over the treatment course, a gradual decline in regulatory T cell populations was observed. This was present in all groups and is likely related to blockade of the CD28-B7 pathway, which is known to be important in maintenance of regulatory T cell homeostasis (18).

Preservation of protective immunity, an important consideration for any immunosuppressive regimen, is of particular concern here. Congenital deficiency of LFA-1 activity is the underlying physiologic mechanism in type-1 Leukocyte Adhesion Deficiency, a severe immunodeficiency syndrome characterized by recurrent non-purulent bacterial and fungal infections. Furthermore, a humanized anti-LFA-1 antibody, efalizumab, was removed from the market following development of Progressive multifocal leukoencephalopathy (PML), a manifestation of reactivation of the JC virus, in patients being treated for psoriasis. Duration of therapy appears to be an important factor in the development of PML while on efalizumab. No cases of PML were reported in the over 2,700 patients treated with efalizumab for psoriasis at the time of its FDA approval, most of whom had received it for only 3 months. In comparison, the four cases that led to the agent’s withdrawal were in patients that had each received the medication continuously for over 3 years (19). A review of the reported cases has estimated the incidence of PML in patients receiving efalizumab for more than 3 years is as high as 1 in 400 (20). A Phase I/II study of efalizumab in human renal transplantation was conducted prior to its withdrawal. In this study, there was an 8% rate of PTLD, all occurring in a group receiving high dose efalizumab and standard calcineurin inhibition (21). This treatment protocol limited LFA-1 blockade to a tapering dose over the first 60 days post-transplant in order to reduce the risk of impaired protective immunity associated with long-term exposure. During the experimental period, we observed repeated episodes of rhCMV viremia in the animals receiving anti-LFA-1 therapy. Previous reports have indicated adhesion molecule blockade, including CD11a blockade, can negatively impact the effect of NK cells on CMV infected cells (22). While some rhCMV reactivation was also seen in the control group receiving belatacept alone, possibly from latent CMV in myeloid progenitors reactivated in response to cytokine (23), the episodes were less frequent and never reached the treatment threshold.
Limiting the exposure to anti-LFA-1 therapy may have also contributed to the failure of this regimen to improve rejection free survival in our renal model. It was noted that the elevation in serum creatinine seen in animals rejecting their graft occurred simultaneously to resolution in the peripheral leukocytosis and tapering of the dose. Similarly, in the clinical islet transplantation trial using efalizumab, graft failure was observed in 2 of 3 patients transitioned to abatacept from efalizumab following its withdrawal from the market (9). Furthermore, psoriatic flares have also been observed in dermatologic patients following cessation of efalizumab therapy (24,25). While it is unclear whether this represents a simple loss of therapeutic benefit versus a true rebound phenomena, the sum of these observations suggest that continued blockade of LFA-1 signaling is required to retain any potential anti-rejection benefit. Thus, while rejection emerged during the tapering period with LFA-1 blockade, indicating that prolonged therapy could perhaps prevent rejection, the impact on protective immunity indicated that prolonged LFA-1 blockade would be poorly tolerated, and that the therapeutic window is too narrow.

The failure of LFA-1 blockade to provide a rejection-free survival benefit when added to belatacept in our renal model is in stark contrast to the previous experience in the islet model. This study was intentionally designed to use an identical dosing regimen. One possible explanation for the difference in outcomes is more rigorous MHC mismatching in the current study utilizing 454 pyrosequencing, which was not available at the time of the prior study. Another possibility is that the dose of anti-LFA-1 antibody used, while sufficient for small volume islet cell infusions, is insufficient for the much larger mass of donor cells present in a solid organ allograft. Lastly, renal transplants are primarily vascularized allografts. Given the importance of LFA-1 in adhesion of leukocytes to capillary endothelium, it is conceivable that the presence of donor endothelium within the graft, particularly that activated by ischemia-reperfusion events, could significantly alter the efficacy of anti-LFA-1 therapies.

The previous report using the islet model also showed LFA-1 blockade to be effective in combination with basiliximab and rapamycin. Furthermore, promising results were seen in clinical pilot studies of efalizumab in various combinations with cyclosporine, mycophenolate, and/or rapamycin in renal and islet transplantation (21,26). In designing this set of experiments, we chose to focus specifically on the role of anti-LFA-1 therapy in costimulation blockade-resistant rejection. Thus, we did not test any combination of TS1/22 with standard immunosuppression. It is possible TS1/22 may provide an additional benefit in that setting.

In this study, we also evaluated the relative efficacy of chimeric forms of the TS1/22 antibody. The primatized versions of the antibody should be less likely to elicit an anti-drug response from the recipient’s immune system, and while the experimental course was truncated by rejection in this study, it is clear that antibody clearance was not a prominent issue in the studies performed. Additionally, belatacept has previously been shown to be very effective in preventing de novo antibody production (27). Any real benefit of chimeric TS1/22 over the fully mouse antibody may be masked by the effect of costimulation blockade on the anti-drug response.

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In summary, short-term use of LFA-1 blockade in combination with costimulation blockade with belatacept failed to prolong graft survival compared to costimulation blockade alone. This study highlights the differences between the pancreatic islet and renal transplantation models in NHPs and supports the later as a more rigorous platform for the study of costimulation blockade-resistant rejection. Further studies would be required to determine whether long-term LFA-1 blockade would be successful in preventing rejection in this setting. However, given the risks of impaired protective immunity observed in this and other studies, caution should be taken without the development of more specific or targeted anti-LFA-1 agents.

Acknowledgments

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Abbreviations

NHP Non-human Primate
LFA Leukocyte Function-associated Antigen
rhCMV Rhesus Cytomegalovirus
PBMC Peripheral blood mononuclear cell
PML Progressive multifocal leukoencephalopathy
T naïve T cell
T_{CM} central memory T cell
T_{EM} effector memory T cell
VLA Very Late Antigen

References


19. FDA Public Health Advisory Updated Safety Information about Raptiva (efalizumab). 2009


Figure 1.
Experimental Plan. All groups received belatacept and methylprednisolone, while only the experimental groups received anti-LFA-1 therapy.
Figure 2.
TS1/22 blocks staining of CD11a on circulating CD3+ cells. At 7 days, all forms of TS1/22 significantly decreased staining for CD11a. This effect lasted until Day 14 for the mouse antibody, and through day 28 for the chimeric forms. * p<0.05, ** p<0.01
Figure 3.
All forms of TS1/22 induce a peripheral lymphocytosis until day 28 (A). This is predominately composed of CD3+ cells (B).
Figure 4.
Relative abundance of memory and naïve CD8+ T cell subpopulations was independent of treatment with TS1/22. (A) Control group receiving belatacept alone. (B) Group receiving belatacept and mouse TS1/22. Similar kinetics were observed in groups receiving the chimeric forms of TS1/22 (not shown).
Figure 5.
Relative abundance of CD4+ regulatory T cells decreased initially and was not affected by anti-LFA1 therapy. Similar kinetics were observed with in groups receiving the chimeric forms of TS1/22 (not shown).
Figure 6.
Serum creatinine levels for all treatment groups: (A) Belatacept control group, (B) Mouse TS1/22, (C) TS1/22 R1, (D) TS1/22 R4.
Figure 7.
Representative histology (20x) at time of rejection from each treatment group: (A) Belatacept control group, (B) Mouse TS1/22, (C) TS1/22 R1, (D) TS1/22 R4.
Figure 8.
The addition of TS1/22 does not prolong graft survival beyond the base regimen.
Figure 9. As the dose of TS1/22 tapers (gray bars), the peripheral lymphocytosis (dashed line, left axis) resolves and creatinine (solid line, right axis) increases. Data shown here is the aggregate for the group receiving TS1/22 R4. Data from the groups receiving the mouse antibody or TS1/22 R1 were similar. Creatinine did not rise in the same time frame in the control group (dotted line, left axis).
CMV reactivations were seen in all treatment groups. Animals receiving LFA-1 blockade had more frequent, and persistent reactivations. 10,000 copies/mL was used as the threshold for conversion from prophylactic antivirals to therapeutic antivirals. No animal developed clinical signs of CMV illness. CMV, cytomegalovirus.
Table 1

Rejection-Free survival for each treatment group.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Rejection-Free Survival (days)</th>
</tr>
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<tbody>
<tr>
<td>Belatacept</td>
<td>42, 70, &gt;385</td>
</tr>
<tr>
<td>Belatacept + Mouse TS1/22</td>
<td>28, 63, 126, &gt;367</td>
</tr>
<tr>
<td>Belatacept + TS1/22 R1</td>
<td>21, 28, 35</td>
</tr>
<tr>
<td>Belatacept + TS1/22 R4</td>
<td>28, 28, 30, &gt;267</td>
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